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Dynamical features in fetal and postnatal zinc-copper metabolic cycles predict the emergence of autism spectrum disorder

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Abstract: Metals are critical to neurodevelopment, and dysregulation in early life has been documented in autism spectrum disorder (ASD). However, underlying mechanisms and biochemical assays to distinguish ASD cases from controls remain elusive. In a nation-wide study of twins, we tested whether zinc-copper cycles, which regulate metal metabolism, are disrupted in ASD. Using novel tooth-matrix biomarkers that provide direct measures of fetal elemental uptake at 2-3-day resolution, we developed a predictive model to distinguish participants who would be diagnosed with ASD in childhood from those who did not develop the disorder. We replicated our findings in three independent studies in the US and UK. We show that three quantifiable characteristics of fetal and postnatal zinc-copper rhythmicity are altered in ASD; the average duration of zinc-copper cycles, regularity with which the cycles recur, and the number of complex features within a cycle. In all independent study sets and in the pooled analysis, zinc-copper rhythmicity was disrupted in ASD cases. In contrast to controls, in ASD cases the cycle duration was shorter ($F=52.25$, $P<0.001$), regularity was reduced ($F=47.99$, $P<0.001$) and complexity diminished ($F=57.30$, $P<0.001$). With two distinct classification models that used metal rhythmicity data, we achieved 90% accuracy in classifying cases and controls, with sensitivity to ASD diagnosis ranging from 85 to 100% and specificity ranging from 90 to100%. These findings suggest that altered zinc-copper rhythmicity precedes the emergence of ASD, and quantitative biochemical measures of metal rhythmicity distinguish ASD cases from controls.

Introduction

Autism spectrum disorder (ASD) affects 1 to 2% of the population in developed countries. Reports have shown that multiple nutrient elements and toxic metals are differentially absorbed and metabolized in children with ASD (*1-3*). However, the mechanisms underlying elemental dysregulation and ASD risk are not well understood, and it is not known if this elemental dysregulation is present in fetal and early postnatal life before first clinical symptoms manifest.

In this study, we tested whether zinc-copper pathways were disrupted in ASD, and if quantitative measures of zinc-copper dynamics could provide a biochemical basis to distinguish ASD cases from controls. We selected zinc-copper cycles as our primary target for testing the dysregulation hypothesis because these are highly conserved in evolution, are essential for maintenance of health, and also exert homeostatic control over known neurotoxicants (*4*). Notable examples of disorders arising from perturbations of the metabolism of these metals include Wilson's disease, a key component of which is copper dysregulation that leads to progressive neurological and cognitive dysfunction and psychosis-like symptoms also documented in adults with ASD (*5*). In Wilson's disease, realigning the zinc-copper interaction via zinc supplementation increases the expression of the metal-binding protein metallothionein that helps regulate copper levels (*6*). Recently zinc-associated pathways have been implicated in ASD (*4, 7, 8*). For example, mutations in the gene coding for *SHANK3*, which is part of a zinc-mediated signaling system, are linked to increased ASD risk (*9*).

Many physiologic processes follow cyclic patterns or rhythms that operate at a wide range of time intervals, from milliseconds (e.g., neuron firing) to hours (e.g., body temperature), days or circadian (e.g., sleep cycles), weeks, or longer (e.g., menstrual cycles) (*10, 11*). For any system in the body, multiple cycles operating at different timescales may interact, and the study of such processes requires methods that focus not solely on concentration, but rather on the dynamic changes over time (*12, 13*). Zinc and copper levels are not stationary in the human body, exhibiting a cyclic pattern (*14-16*). We, therefore, focused on their cyclic properties rather than single point measures of serum concentrations. We use the term 'cycles' to indicate time-dependent rhythmic variation in the concentration of elements. Our methodology samples elemental concentrations in incremental zones of teeth (akin to growth rings in trees) at approximately 2 to 3 day intervals, which allows the identification of cyclical processes varying at an approximately 7- to 10-day period. Contrasting these cycles between ASD cases and controls offers a way to uncover system-wide dysregulations in zinc-copper rhythmicity. Furthermore,

because zinc and copper pathways are central regulators of multiple metals, their disruption would have downstream effects including incomplete metabolism of other essential elements and toxic metals (*17, 18*). We, therefore, also studied the metabolic cycles of other metals during the fetal and early childhood periods (from the second trimester to approximately one year of age) (*19, 20*). To test our hypothesis, we applied the tooth biomarkers in four case-control samples; a discovery population of twins from Sweden and replication in two US case-control samples and a birth cohort from the UK.

Results

Dysregulation of Zinc-Copper Cycles in ASD

Our primary hypothesis predicts dysregulation of zinc-copper cycles in ASD, as quantified by cycle duration, complexity, and regularity. The mean diagonal length (MDL) measures the duration of a cycle and was used in our study to detect fragmentation of zinc-copper cycles (Fig. 1 and Supplementary Methods). We found that MDL for zinc-copper cycles was significantly reduced across all cohorts ($F = 52.25$, $P < 0.001$), reflecting an average reduction of 15% in ASD twins (Fig. 2A). We also detected a significant ASD*Study interaction ($F = 3.83$, $P = 0.014$), reflecting some across-study variability in the magnitude of ASD-related reductions, but post-hoc tests confirmed significantly reduced MDL in ASD cases in all populations studied (Sweden, $F = 38.05$, $P < 0.001$; New York, USA, $F = 8.07$, $P = 0.029$; Texas, USA, $F = 12.41$, $P = 0.005$; UK, $F = 8.57$, $P = 0.023$). For additional details see Table S1.

Zinc-copper cycles in ASD cases also exhibited significantly reduced entropy (a measure of biochemical network complexity) in contrast to non-ASD controls, who had more complex cycles ($F = 57.3$, $P < 0.001$, Fig. 2B). After correction for multiple comparisons, we found no evidence that this effect varied across populations; that is, the attenuation of entropy in ASD cases was consistent across studies.

Finally, we found that determinism, a measure of cycle regularity and resistance to perturbation was significantly reduced in ASD patients ($F = 47.99$, $P < 0.001$, Fig. 2C). As with entropy, we found that the ASD-related attenuation of determinism was consistent across populations.

Accuracy of Elemental Dynamics for Classifying ASD

Given the significant differences we observed between ASD cases and neurotypically developing controls across multiple dynamical features of elemental cycles, we implemented an ensemble machine learning classification method generalized from weighted quantile sum (WQS) regression (*21*) to gauge how the full range of cyclical features classify subjects as ASD cases or controls independently of their population of origin. Using optimal threshold criteria, this model was 90% accurate in predicting ASD cases, with 100% sensitivity for ASD diagnosis, 85% specificity to controls. Fig. 3A shows the classification performance of this model as applied to the holdout dataset (15% of data). To confirm that robust classification could be achieved independently of the predictive algorithm applied, we also implemented an alternative approach based on penalized logistic regression (LASSO). This approach achieved similar overall model accuracy (90%; model performance summarized in Fig. 3B), with slightly less sensitivity but improved specificity. Model performance relative to WQS is summarized in Fig. 3C; additional details on the construction of classification algorithms in presented in the Supplementary Materials.

Discussion

We tested the hypothesis that disruption of zinc-copper dynamics underlies the elemental dysregulation that has been observed in ASD (*1-3*). We used tooth matrix biomarkers to measure detailed temporal profiles of zinc, copper and other elements from the second trimester to approximately one year postnatally. To characterize the co-progression of zinc and copper cycles throughout early development, we used cross-recurrence quantification analysis (CRQA), a mathematical approach that originates in dynamical systems theory. We identified cycles varying on an approximately 10-day period, and found strong evidence for abnormalities in zinc-copper cycles in ASD characterized by shorter duration, lower complexity and less determinism. Further, we found that the integration of these dynamical features in an ensemble machine-learning algorithm, allowed a robust classification of ASD cases and controls, which we were able to replicate with similar accuracy with an alternative predictive algorithm based on penalized logistic regression. Taken together, these findings suggest that the normally well-coordinated and intricate metabolism of these essential elements is fragmented in ASD.

Metals have long been implicated in autism (*1-3*). Early life exposure to toxic metals and deficiencies of nutritional elements have been linked with several adverse developmental outcomes frequently associated with ASD, including intellectual disability, and language,

attentional and behavioral problems (*22*). Animal studies show that the effects of various metals on brain development could be mediated through dysregulation in neurotransmission, and alterations in frontal and subcortical brain structures (*23*), which have also been implicated in ASD (*24*). Here, we found that measures of metal rhythmicity were associated with ASD case status, but not metal concentrations. These data emphasize that the dysfunctions apparent in cyclical properties are not evident in raw measures of concentration, which highlights the utility of our approach. This does not suggest that elemental concentrations are not relevant to the etiology of ASD, as we have previously published findings linking elevated levels of several elements (*25*). That study dealt exclusively with a twin sample, however, and without the inherent control of genetic and environmental confounds in non-twins, those differences in elemental concentrations may not be apparent.

Dynamic rhythms are central to human health and disease, and have been reported in many systems (*12, 13*). Notably, much research has been done on imprints of rhythms in mammalian teeth (*26, 27*), making naturally shed deciduous human teeth an ideal biomatrix to study the role of metal rhythmicity and its disruption in childhood disorders, as we have done here for ASD. A key feature of our approach is the focus on dynamic rhythms in the internal metabolism of essential elements and toxic metals, rather than solely relying on an exposure paradigm that emphasizes external environmental exposures. By doing so, we could identify disrupted zinc-copper (and zinc-lead) cycles, even in populations where exposures were not excessive. In contrast to prior studies that have employed blood or urinary biomarkers to investigate cyclical elemental dynamics in humans over periods less than a week, our methods allowed a longitudinal analysis over a prolonged developmental period extending from the second trimester to approximately 1 year of age. An important consideration is that these differences in the developmental periods studied and temporal resolution between our study and those utilizing repeated blood or urine measurements, do not imply that the underlying processes being observed are dissimilar or unrelated; rather, we suggest that the cyclical processes we capture are superimposed on daily oscillations that have been previously described (*14, 15*). Independent of the duration of the cycles studied, we consistently found dysregulated cycle complexity and regularity in ASD cases across multiple replication samples, which bolsters our results against random measurement errors, and served as the basis of our classification analysis.

Critically, the focus on elemental dynamics represents a novel theoretical perspective in the consideration of factors predictive of ASD. While the role of elemental exposures in ASD is well-

studied, previous investigations have considered primarily on the role of exposure intensities, i.e. biomarker concentrations. Our results provide a new perspective, in that the temporal organization of elemental assimilation, and the interaction of multiple elemental pathways, appears critical to the emergence of ASD. Importantly, this theoretical perspective, which we refer to as systemic elemental dysregulation (SED), opens new avenues for investigating elemental dysregulation in ASD and other disorders, and yields testable hypotheses for future investigations to pursue. For example, our finding of dysregulated zinc-copper rhythmicity suggests underlying dysregulation in mechanisms involved in zinc-copper metabolism, which will be pursued in future studies with in vivo animal models and in vitro induced pluripotent stem cells (iPSC), where experimental manipulation of zinc-copper dynamics is possible. Consistent with this, a recent study (*28*) identified zinc deficiency and attenuated zinc transporter expression in humans with autism-related mutations in the SHANK3 gene, consistent with the SEDs etiological perspective of dysregulated metabolic processes (*3*). Additionally, our results emphasize the need to investigate other diseases where elemental assimilation is known to play a role, for example ADHD, but has to-date been primarily studied through the lens of exposure intensity.

We also studied other elements because disruption of zinc and copper cycles may have downstream effects including incomplete metabolism of other elements and toxic metals (*17, 18*). While we did not directly test biochemical pathways underlying interactions between zinc, copper and other elements, we observed alteration in the joint rhythmicity of zinc and lead characterized by smaller and less complex cycles (Fig. S1). Shorter and lower entropy interactions suggest that the regulatory effect of zinc on toxic metals, which is generally perceived as protective, is perturbed. We also found differences in other metals between cases and controls that were restricted to some but not all study samples (see Tables S1 and S2). This may be due to population-specific dynamics, such as differences in diet or exposure to pollution, which we have not analyzed in-depth here.

Strengths of this study include the use of recently developed and validated methods to measure metals in teeth that provide detailed temporal profiles of elemental uptake during the fetal and early childhood periods. The use of population-derived discovery cohorts and the fact that our results could be replicated in ASD children from several geographical regions further bolsters the validity of our findings. Limitations include small discovery and replication populations, but together our sample size is approximately 200 participants, which was sufficient to detect and

replicate significant elemental disruptions. Nonetheless, it is possible that other elements are also affected, which would only be detected with larger sample sizes. Our laser-based sampling method did not provide sufficient sampling points to separately study metal rhythmicity during the prenatal period from that observed after birth. Given that metal metabolism processes can incorporate toxic elements with similar chemical properties (cadmium, mercury among others), the dysregulatory mechanism described herein may also play a role in their well-described neurotoxicity. Furthermore, other neuroactive metal regulatory pathways such as iron and manganese deserve study as well.

In conclusion, in a discovery set of twins, including monozygotic twins discordant for ASD, and three independent replication population samples, zinc-copper cycles were consistently altered in ASD. Furthermore, quantitative biochemical measures of the joint cyclic properties of zinc and copper accurately distinguished ASD cases from controls.

Materials and Methods

Study participants

Our participants were recruited from four different studies being undertaken in three countries. Key characteristics of the studies are outlined in Table 1 and details of participant recruitment and ASD case ascertainment are provided in the Supplementary Methods. In brief, a discovery analysis was undertaken in the Roots of Autism and ADHD Twin Study in Sweden (RATSS), which is designed to investigate the genetic and environmental determinants of ASD. Twins are recruited from nationwide registries and by advertisements in Sweden (*29*). From the whole RATSS cohort, we collected and analyzed teeth from 75 participants (32 complete twin pairs and 11 individuals from twin pairs whose sibling did not donate a tooth). This subsample constitutes 26% of the RATSS cohort, and 50% of all participants in RATSS of tooth shedding age.

For our primary replication analysis, we obtained teeth and supporting data from 25 ASD cases and 25 gender matched controls enrolled in a long-term pregnancy cohort in the UK - the Avon Longitudinal Study of Parents and Children (ALSPAC) (*30*). We undertook additional replication analysis by including ASD diagnosed individuals (N = 10) at an autism clinic in New York, USA, and their unaffected siblings (N = 8) (*31*). Finally, we enrolled a random sample of 25 cases and 25 controls from a community-based study in Texas, USA – the Autism Tooth Fairy Project – that collects teeth and supporting data from parents through a community network (*32*).

Laboratory Assessments

Details of tooth collection protocols of each study population are provided in the Supplementary Materials. Our approach to measuring metals in teeth using laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS) and assigning developmental times has been detailed elsewhere **(33, 34)**. Briefly, teeth are sectioned and the neonatal line (a histological feature formed in enamel and dentine at the time of birth) and incremental markings are used to assign temporal information to sampling points. A New Wave Research NWR-193 (ESI, USA) laser ablation unit equipped with a 193nm ArF excimer laser was connected to an Agilent Technologies 8800 triple-quad ICP-MS (Agilent Technologies, USA). Helium was used as a carrier gas from the laser ablation cell and mixed with argon via Y-piece before introduction to the ICP-MS. The system was tuned daily using NIST SRM 612 (trace elements in glass) to monitor sensitivity (maximum analyte ion counts), oxide formation (232Th16O+/232Th+, < 0.3%) and fractionation (232Th+/238U+, 100 ± 5%). The laser was scanned in dentine parallel to the DEJ from the dentine horn tip towards the tooth cervix. A pre-ablation scan was run to remove any surface contamination. Data were analyzed as metal to calcium ratios (e.g. 208Pb:43Ca) to control for any variations in the mineral content within a tooth and between samples. On average, each tooth was sampled at 152 locations, reflecting a sampling window for metal uptake of -113 to 214 days since birth, on average. LA-ICP-MS operating parameters are given in Table S3.

Statistical analyses

Traditional statistical approaches to uncovering the association of environmental stressors and disease outcomes, such as regression modeling, use concentrations at given time points as the fundamental units of analysis, but do not resolve the dynamic cyclical nature of environmental exposures and their metabolism. To overcome this limitation, we used recurrence quantification analysis (RQA) and cross-recurrence quantification analysis (CRQA), which are non-linear analytical methods for studying cyclical signal properties. These methods are well-characterized with applications in diverse scientific fields, including physiology, molecular biology, geophysical sciences, and psychology; for reviews, see Webber and Zbilut (*35, 36*) and Marwan et al. (*37*). The methods applied here, summarized in Fig. S2, are described at length in Curtin et al. (*38*) and were implemented via the Cross-Recurrence Toolbox v5.16 (*39*) in Matlab v2016b (Mathworks). Briefly, for RQA, time-series metal data from deciduous teeth were first used to construct recurrence plots (RPs), with delay (τ) and embedding-dimension (m) parameters calculated by the minimization of mutual information and false nearest- neighbors' algorithms,

respectively (Fig. S2). To facilitate cross-subject comparisons, threshold functions, ε, were constrained to fix signal recurrence rates to 10%. The temporal organization of features in the resulting RPs was then quantified via RQA to yield measures of mean diagonal length (MDL), recurrence time (RT), determinism, and entropy. These measures capture different features of diagonal line structures in RPs, which reflect cyclical signal components. MDL quantifies the average duration of these cycles, while RT measures the average interval between cycles. Determinism compares the ratio of cyclical to non-cyclical recurrence points, and is therefore a unit-less metric akin to a percentage, while entropy, a unit-less metric of predictability, measures the variability in cyclical lengths to capture the complexity of periodic signatures. For CRQA, these methods are extended to cross-recurrence plots (CRPs), which capture the temporal organization of two signals cyclical interactions over time (*35-37*).

Prior to inferential statistical tests, all variables were evaluated to confirm assumptions of normality in variable distributions. For analyses of recurrence features (RQA/CRQA), linear mixed models were used to test main effects of ASD-diagnosis on features while also testing for potential interactions of ASD-diagnosis and study population to evaluate population-specific effects. Familial relationships among subjects, including siblings and twins, were modeled as random variables to account for non-independence of twins and siblings, while also controlling for sex as a covariate. Additional covariates, including zygosity, gestational age, birth weight, and diagnosed comorbidities, including ADHD and intellectual disability, were also initially modeled, but these were ultimately excluded as they caused only negligible adjustments in our primary predictors and had no significant associations with outcomes. *P* values for study-specific differences reflect post-hoc analyses of ASD*Study interactions with Bonferroni adjustments for multiple comparisons. *P* values describing differences in the overall pool of subjects reflect main effects of ASD diagnosis with false discovery rate (FDR) *P*-value adjustments.

Two classification models were undertaken; a generalization of weighted quantile sum (WQS) regression (*21*) to a binomial distribution to create a logistic classification algorithm, or the application of penalized logistic regression (LASSO) (*40*). Briefly, for WQS, data from all study populations were pooled and then divided into training (34% of sample size), validation (51%), and hold-out (15%) subsets. The training dataset was analyzed via WQS regression, yielding an empirically-weighted index characterizing the mixture of recurrence variables, a beta parameter estimating the association of this weighted index with ASD diagnosis, and an index of weights

describing each individual variable's contribution to the overall index. Weights derived from the training set were then used to construct and test a WQS index in the validation dataset, and the resulting beta estimates for the index were used to calculate a regression model for predicting outcomes in the holdout dataset, which was entirely naïve to estimation of either weights or regression parameters. We randomly selected participants from all the populations for the classification analysis so that no single study population can drive the classification accuracy (or specificity or sensitivity); and, significant features would be predictive independently of population.

For predictive models based on penalized logistic regression, data were initially randomly subset into training (85% of participants) and holdout (15% of participants) datasets. As in the implementation of WQS, all measures derived from RQA (mean diagonal length, entropy, determinism, recurrence time) for all elemental pathways and cross-recurrences studied were included in model building. Ten-fold cross validation was then applied in the training dataset to identify a model with minimal deviance. The optimal LASSO model was then used to predict case/control status in the naïve holdout dataset. Additional details on model formulation and implementation are provided in the Supplementary Materials.

In evaluating the performance of predictive classifiers, sensitivity was calculated as true positive predictions / number of positive cases; specificity was calculated as true negative predictions / number of negative cases; and, accuracy was calculated as the number of correct predictions / number of total predictions. We confirmed the suitability of undertaking a pooled analysis by establishing the equivalence of the control subjects across the four study populations (see Fig. S3, and Bioequivalence Testing in Supplementary Materials).

Supplementary Materials

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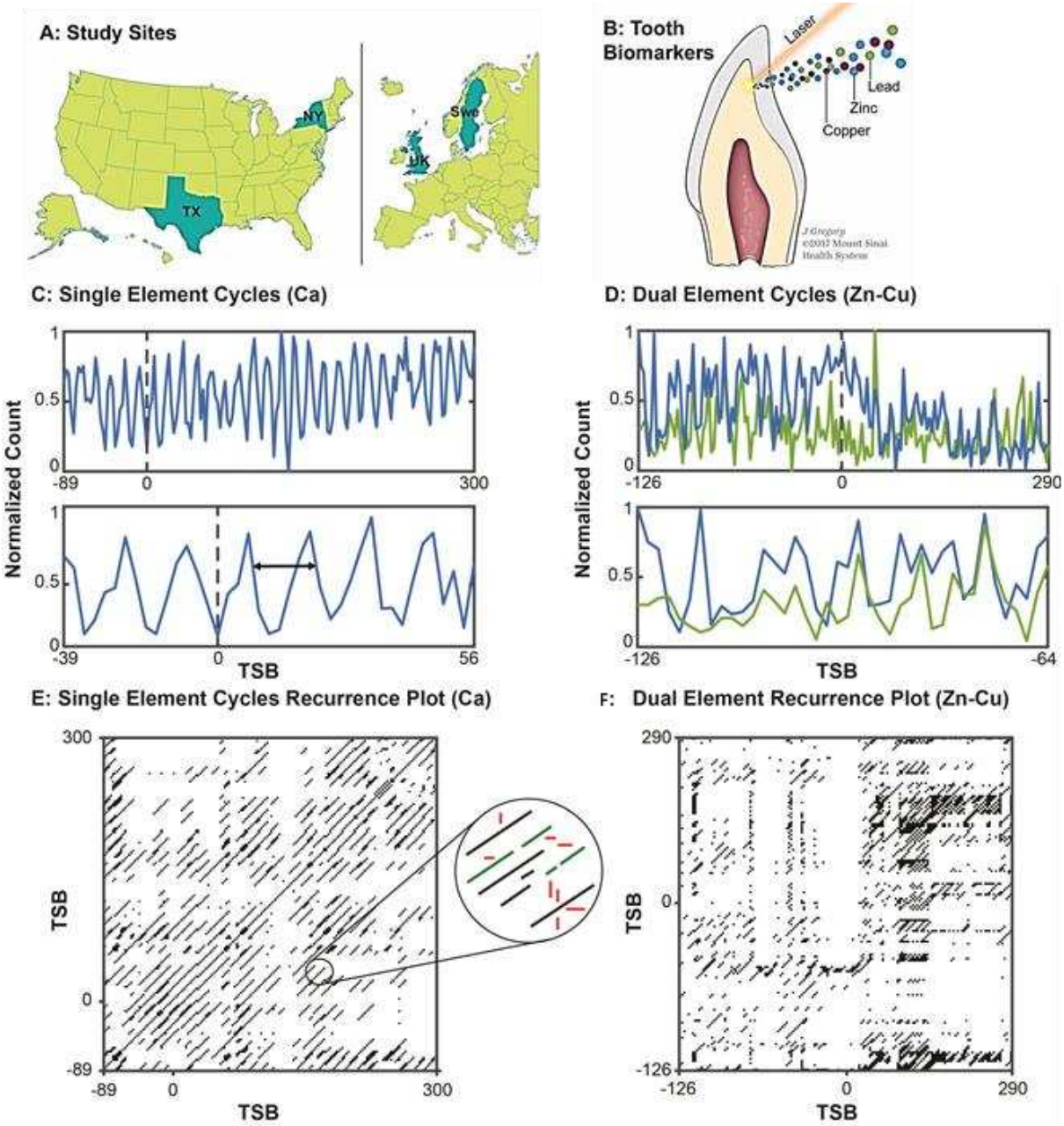
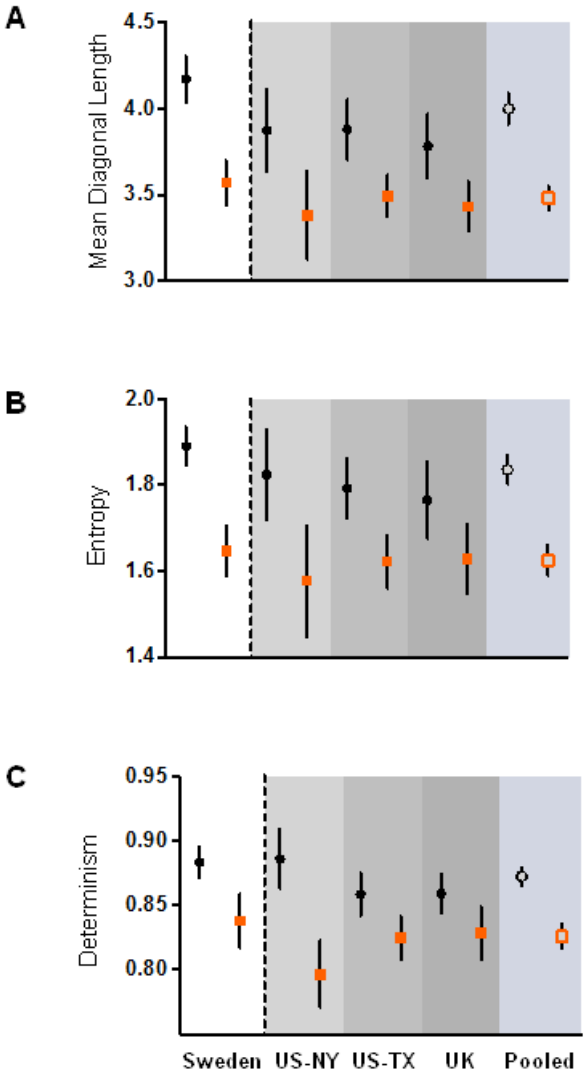
Fig. 1. Overview of Study Design.



(**A**) Study participants recruited from Sweden (twin - co-twin discovery set), and from the US and UK (case-control replication sets). (**B**) Collected deciduous teeth were analyzed using laser ablation-inductively coupled plasma-mass spectrometry to generate temporal profiles of metal uptake during fetal and postnatal development. (**C**) Example exposure profile in one subject (top panel) ranging from -89 to 300 days since birth; dashed line indicates birth, while black arrows indicates a period of approximately 10 days. Bottom panel shows magnified region from -39 to 56 days since birth (TSB; time since birth in days) to highlight cycles in elemental concentration varying on a roughly 10-day period. (**D**) In top panel, example elemental exposure profiles in a single subject for two elements (Zn, blue line; Cu, green line) simultaneously sampled and overlaid from -126 to 290 TSB. Bottom panel shows magnified region from -126 to -64 TSB showing concentration of both elements rising and falling in synchrony. (**E**) Recurrence plot generated from single element trace in Panel C. This graphical analytical tool, analogous to a spectrogram, presents cyclical processes as diagonal lines to allow the timing and distribution of

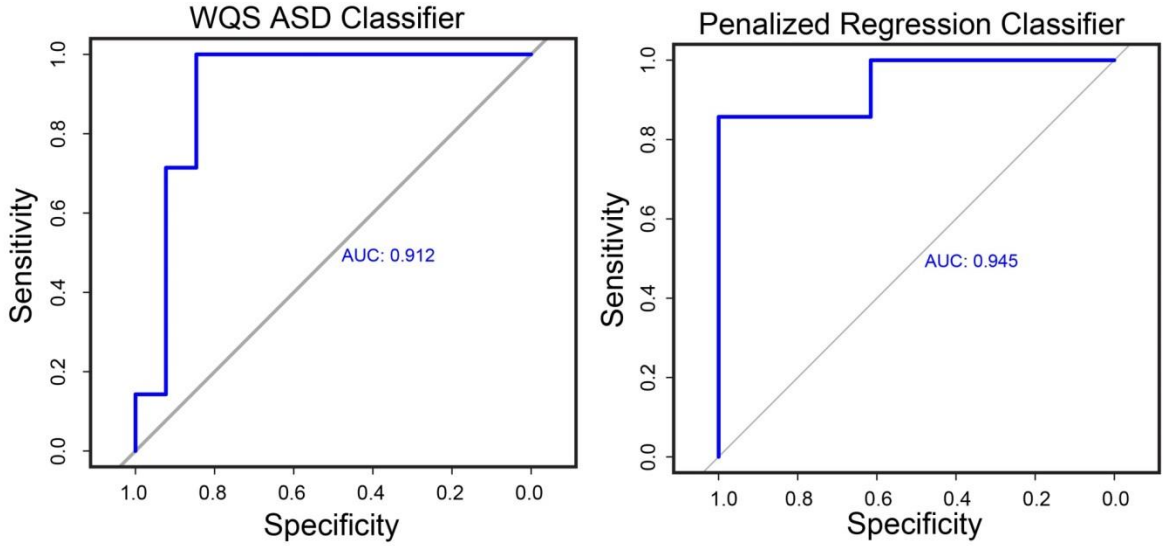
cycles to be analyzed, and contrasted with singular moments that do not repeat (represented as white space), points that recur only once (singular black points), or periods of stability where concentrations are relatively constant over time (vertical or horizontal lines); these structures are emphasize in the circular inset. During recurrence quantification analysis (RQA), the duration of cycles is captured by measuring mean diagonal length (MDL), robustness (determinism), and complexity (entropy). (**F**) Cross-recurrence plot (CRP) for the dual element cycles presented in Panel D.

Fig. 2. Disruption of Zinc-Copper Cycles in ASD Cases and Controls.



Mean diagonal length (**A**), entropy (**B**) and determinism (**C**) are reduced in autism cases (squares) compared to controls (circles) in all study populations, indicating that zinc-copper cycles are of shorter duration, lower complexity and reduced regularity in cases. Pooled estimates generated by combining data from all studies. Data are means +/- 95% confidence intervals.

Fig. 3. Performance of WQS-Regression and Penalized Logistic Regression Algorithms in Classifying ASD Cases and Controls.



Classification Method	Validation Strategy	Model AUC	Model Accuracy	Model Sensitivity	Model Specificity
WQS	Training, Validaton, Hold-out	0.912	90%	100%	90%
Penalized Logistic Regression	10-fold Cross-validation	0.95	90%	85%	100%

(**A**) ROC curve showing classification performance of the WQS algorithm with varying threshold values applied to the holdout dataset (15% of data). AUC, area under the curve. (**B**) Classification performance of a penalized logistic regression algorithm applied to a holdout dataset (15% of data) following 10-fold cross-validation in a training dataset (85% of data). (**C**) Model performance characteristics.

Table 1. Characteristics of Study Participants.

Study	Location	Design	N (cases)	Male:Female	Mean gestational days (SD)
RATSS	Stockholm, Sweden	National prospective twin cohort	75 (20)	46:29	247 (24)
ALSPAC	Bristol, UK	Case-control nested in prospective cohort	50 (25)	36:14	271 (21)
Seaver Autism Center	New York, USA	Hospital based case-control	18 (10)	12:6	258 (21)
Autism Tooth Fairy Study	Texas, USA	Community based case-control	50 (25)	25:25	N/A

Supplementary Materials

Materials and Methods

Study Populations

We undertook this study in four geographically distinct populations. An important consideration for our discovery analysis was to apply control over genetic factors, which we achieved by comparing individuals with ASD with their discordant twin siblings and non-ASD twins in the Roots of Autism and ADHD Twin Study (RATSS). However, after discovery, we considered it important to extend our findings to non-twin siblings and unrelated participants. We also preferred to include participants from different geographic regions, rather than undertake replication in a single study. To achieve this aim, our primary criteria for inclusion were that children enrolled in established studies with an ASD diagnosis that required treatment by a medical professional, were able to provide naturally shed deciduous teeth, and had no known neurodevelopmental disorder or genetic syndromes. Since our analysis does not include cross-population comparisons of diagnostic indices, we included children diagnosed with any validated clinical diagnostic tests for ASD. Our study has 75 discovery participants, and an additional 118 participants for our replication analysis (Table 1).

Roots of Autism and ADHD Twin Study (RATSS). Description of the RATSS for projects using teeth has been reported before (*1*). Participating twins in this study are part of RATSS recruited between 2011 and 2016 (*2*). The study was approved by the Swedish Regional Ethical Review Board and all participants gave written informed consent. Potential twin participants for the RATSS are identified through nationwide registries, including the Child and Adolescent Twin Study in Sweden (CATSS) (*3*), a population-based study of all twins born in Sweden since 1992 in which all twins are screened at age nine using the Autism, Tics, ADHD and other Comorbidities Inventory (A-TAC). Participants are identified through linking the Swedish Twin

Registry to other National registries such as the Swedish National Patient Register, and regional clinical registers in Stockholm County (Child and Adolescent Psychiatry ["Pastill"], Habilitation & Health Centers) that include ICD-10 diagnostic information (*4, 5*). Finally, potential participants are also identified through Swedish societies for neurodevelopmental disorders (NDDs) as well as advertisements and summons in the media. Even though the recruitment is done through different routes, >80% of the twins in RATSS are present in the Swedish twin registries.

Twin pairs were recruited into the RATSS either based on discordance for ASD (>2 points differences on the A-TAC autism subscale equaling ~1 SD); concordance for ASD (both twins reaching cut-off on the A-TAC autism scale); or concordance for no NDD (both twins under cut-offs for all NDD subscale on the A-TAC). For other sources of recruitment, the twins are invited if at least one twin has an ICD-10 diagnosis of autism (F84.0), Asperger syndrome (F84.5), or atypical autism/pervasive developmental disorder not otherwise specified (PDD-NOS) (F84.1, F84.8, F84.9), or a Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5) diagnosis of ASD (either parent- or registry reported). All potential participants undergo a telephone interview by a research nurse checking eligibility before the invitation for assessment in RATSS. Zygosity was determined by genotyping of saliva, or whole-blood derived DNA using standard methods. The genotyping was done using Infinium Human-CoreExome chip (Illumina Inc. USA). The estimating identity by descent was analyzed using the PLINK software (v1.07) (*6*) after quality control and removal of SNPs with minor allele frequency less than 0.05 within the samples. All pairs of DNA samples showing $\hat{\pi}$ >=0.99 were considered as monozygotic pairs. For few pairs, a short tandem repeat kit (Promega Powerplex 21) was used to determine the zygosity.

Ascertainment of ASD Cases and Controls: Medical history and sociodemographic information of the families were collected. ASD was diagnosed according to DSM-5 criteria based on clinical experts consensus and corroborated by results from the Autism Diagnostic Interview – Revised (ADI-R) (*7*) and the Autism Diagnostic Observation Schedule Second Edition (ADOS-2) (*8*). Clinical severity of ASD symptoms was determined by ADOS comparison scores, and autistic traits were measured by parent reported Social Responsiveness Scale-2 (SRS-2) (*9*) total raw scores. General cognitive ability was assessed using the Wechsler Intelligence Scales for Children or Adults (Fourth Editions) or the Leiter Scales and the Peabody Picture Vocabulary Test (Third Edition) in cases of low verbal abilities.(*10-12*) Our study included both identical/monozygotic twins and fraternal or dizygotic. There were 34 MZ twins (17 pairs). Additional details have been published elsewhere (*1*).

Tooth Collection and Storage: Parents/guardians collected the naturally shed deciduous teeth at home. Teeth were brought to the study team in person and stored at room temperature until analysis.

Avon Longitudinal Study of Parents and Children (ALSPAC)

ALSPAC is a longitudinal cohort study which recruited 14,541 pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992. 14,541 is the initial number of pregnancies for which the mother enrolled in the ALSPAC study and had either returned at least one questionnaire or attended a "Children in Focus" clinic by 19/07/99. Of these initial pregnancies, there were a total of 14,676 fetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. The number of new pregnancies not in the initial sample (known as Phase I

enrolment) that are currently represented on the built files and reflecting enrolment status at the age of 18 is 706 (452 and 254 recruited during Phases II and III respectively), resulting in an additional 713 children being enrolled. The phases of enrolment are described in detail (*13*). The total sample size for analyses using any data collected after the age of seven is therefore 15,247 pregnancies, resulting in 15,458 fetuses. Of this total sample of 15,458 fetuses, 14,775 were live births and 14,701 were alive at 1 year of age (study website contains details of all data available: http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/) (*13, 14*). Regular data collection is ongoing since September 6th 1990. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees (listed at http://www.bristol.ac.uk/alspac/researchers/research-ethics/). All participants provided written informed consent.

Ascertainment of ASD Cases and Controls: The identification of ASD cases in ALSPAC is described in detail elsewhere (*15*). Briefly, information of ASD diagnosis was obtained from UK National Health Service (NHS) and education sources. The use of multiple sources was implemented to ensure as complete an ascertainment as possible (*16*). All ALSPAC cohort members who had a World Health Organization's International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) diagnosis relating to any form of developmental delay for the period 1991 to 2003 inclusive, or those who were identified in the NHS Child Health computer system as having special educational needs during the same time period were identified, and their health records were obtained from the NHS.

All the NHS Trusts in the ALSPAC area have specialist autism teams in children's services, trained to use standardized assessment tools including the Diagnostic Interview for Social and Communication Disorders (*17*); the Autism Diagnostic Observation Schedule (*18*), and the

Asperger Syndrome Diagnostic Interview (*19*). A team of three experienced researchers searched NHS hospital medical records (in-patient and outpatient) and community child health records (including child development team records) to identify children who had a diagnosis of ASD made after a multidisciplinary assessment. The researchers searched for ICD-10 diagnoses according to a structured proforma, based on information that was available in the records written by the multiprofessional team involved in the care of the child. A board-certified pediatrician with 30 years of clinical experience reviewed all information collected from the notes and confirmed that the diagnostic information was consistent with ICD-10 criteria.

In addition, the Department of Education for England Pupil Level Annual Schools Census (PLASC) dataset which included data on children attending state schools who were recorded as having some form of special educational needs was searched for ALSPAC children with ASD listed as either a primary or a secondary concern.

Tooth Collection and Storage: Teeth were collected when participants were 5-7 years old. Mothers were asked for any teeth shed naturally or extracted by a dentist, without cavities or fillings, to be sent to the ALSPAC team. Mothers were asked to keep teeth at room temperature and to indicate the date when the tooth was shed. Approximately 70% of those contacted provided teeth, and those were kept at -20ºC. For this study, teeth samples from 25 ASD cases and 25 healthy controls with no documented developmental, learning or psychiatric disorder were obtained for the present study. Cases and controls were matched for sex and birth weight.

Seaver Autism Center, New York, USA

The Seaver Autism Center is located at the Icahn School of Medicine at Mount Sinai in New York City and serves a diverse and complex patient population. The Seaver Center has

longstanding community ties and receives approximately 600 new autism referrals annually for research and/or clinical services. Ethical approval for the study was obtained from Mount Sinai School of Medicine research ethics committee. All participants and/or their parents provided written informed consent.

Ascertainment of ASD Cases and Controls: In 2016 we contacted all families in ongoing studies and services at the Seaver Center that (1) had a child with ASD as well as an additional child without a diagnosis of ASD, and (2) the case and sibling where of teeth-shedding age (ages 5-10). Informed consent was obtained from all parents or legal guardians. ASD diagnosis was confirmed through a gold-standard evaluation including the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) (*20*), and the Autism Diagnostic Interview-Revised (ADI-R)(*21*) and a clinical evaluation with a board-certified child and adolescent psychiatrist or licensed clinical psychologist to assess DSM-5 (*22*) criteria for ASD.

Tooth Collection and Storage: Caregivers were provided with an individual collection kit and a corresponding questionnaire for each tooth. The questionnaire included the following multiple choice questions: (1) How was the tooth obtained? Response options: Lose and fell out naturally, removed by a dentist, unknown, other; (2) How was the tooth stored? Response options: Dry, in a plastic bag, in water, in another liquid, unknown, other. Parents were asked to specify when "other" was selected. Demographic information was provided by caregivers for all probands and siblings. These included information on gestational age, birth weight, significant perinatal medical history, and psychiatric diagnoses. Teeth samples from 10 ASD cases and 8 of their siblings with no documented developmental, learning or psychiatric disorder were obtained for the present study. This sample was predominantly white American (77%).

University of Texas Health Sciences Center, Houston, Texas, USA

In January 2011, deciduous tooth collection was commenced in a national sample of families enrolled in the Interactive Autism Network (IAN). The IAN network is the nation's largest online autism research forum where over 43,000 families complete comprehensive surveys and participate in various research studies. Inclusion criteria for IAN participants are parents with children under 18 years of age who have been diagnosed with ASD by a health professional. Twenty-five cases and 25 gender-matched controls were selected for inclusion in this study.

Ascertainment of ASD Cases and Controls: The diagnosis of ASD in the IAN database has been clinically validated in a subsample of participants[89] as well as verified by a review of parent- and professional-provided medical records[90]. This work reports 98% concordance of validated ASD diagnoses with parental self-report, thus supporting the viability of the centralized database recruitment model. Notwithstanding, to verify diagnosis in our non-IAN recruits, a certified diagnostician recently conducted an ADIR interview by phone in a random subset of our participants. We found that there was a 100% concordance between parent-reported and diagnostician assessed ASD. In the current study, we exclude the use of teeth from children whose mothers report having children with various other medical or neurological conditions including ADHD, and retain only those with ASD or those that are typically developing controls with no reported medical, emotional or psychological comorbidities.

We have recruited unrelated population controls through television news spots, print media advertisement, as well as a wide consortium of community-based partners that include several children's dental and well-child medical clinics. Similar to case mothers, control mothers who choose to participate are asked to submit one or more teeth from each of her children. While a

potential source of controls might be obtained from families who are friends of case families, this is not recommended in case-control studies due to unknown inherent confounding biases.[88]

Tooth Collection and Storage: Potential participants contact our research coordinator who then screens them for eligibility. Each mother must be the biological mother of the child whose deciduous teeth will be provided. If eligible, families receive a study packet in the mail consisting of a consent form, tooth envelope, return envelope and a survey. The survey asks information about family demographics, medical history, pre and postnatal care and brief exposure histories. For families with more than one child, separate surveys and shipping packets are mailed.

When the material is returned (we currently have a 90 % return rate from eligible families requesting a packet), each survey and tooth is then given a unique study identification number that links surveys to teeth. Both teeth and survey data are entered into our database and stored in locked file cabinets until they are selected for analysis. For both case and control teeth, we utilize canine, molar and incisor teeth without cavities or fillings. We matched the case and controls in this study by gender and approximate age (within a 5-year range). Teeth are stored at room temperature until analysis.

Bioequivalence Testing

For bioequivalence testing of RQA/CRQA measures of control subjects across populations, we extended standard equivalence testing methods following the principles of confidence interval inclusion[14]. These provide confidence intervals (CIs) on the comparative ratios of population means for a given measure, with equivalence inferred from containment of family-wise CIs within a pre-defined equivalence bound; here, as per FDA guidelines (FDA-CDER, 2003)(*23*),

ranging from 0.80 - 1.25. Asymmetric confidence intervals were constructed by back-transforming confidence intervals from Dunnett's test (with family-wise 95% confidence coefficient, with the discovery cohort as reference) on the difference of logs constructed using least square means adjusted for covariates (here, sex) in a general linear model of each log-transformed measure. Analyses were conducted using PROC GLM in SAS (v.9.4).

Statistical Models

Linear mixed models were used to test for global and population-specific effects of ASD diagnosis on dynamical features measured with RQA, e.g. mean diagonal length (MDL), entropy, determinism. These models followed the equation: $y = X\beta + Zu + \varepsilon$, where y is an RQA feature (e.g., MDL), Xβ reflects fixed effects and associated observations, Zu reflects a design matrix and associated random effect to account for the relatedness, and therefore potential non-independence, of subjects (twins, siblings, or unrelated), with ε as a vector of random errors. The primary fixed effects relating to our study question were ASD (diagnosis as case or control), to test for global (across study) differences in ASD, and an ASD*Study interaction, to test for population-specific differences in ASD, with sex included as a covariate. Additional covariate variables, including zygosity of twins, diagnosed comorbidities, gestational age at birth, birth weight, and diagnosed comorbidities were initially modeled but were ultimately excluded, as these caused only negligible adjustments in associations with our primary predictors and had no significant associations with outcomes. For all models, *P* values related to main hypotheses (ASD effect, ASD*Study interaction) are reported following FDR adjustment for multiple comparisons; these are reported in Table S4. Where study-specific post-hoc tests were conducted, all *P* values reported reflect model-specific Bonferroni adjustment.

For classification with penalized logistic regression, a LASSO model was implemented as per Tibshirani (*24*). The objective function for this was:

$$\min_{(\beta_0,\beta)} \left[\frac{1}{N} \sum_{i=1}^{N} y_i \cdot (\beta_0 + x_i^T \beta) - \log(1 + e^{(\beta_0 + x_i^T \beta)}) \right] + \lambda \|\beta\|_1$$

A selection parameter, α, was fixed at 1 for the LASSO procedure, with outcome (ASD case or control) *y*, model intercept β_0, shrinkage parameter λ, $x_i^T \beta$ the matrix of predictor variables and associated beta estimates, and $\|\beta\|_1$ the L1 norm regularization term. The predictor variables in the model included all RQA features derived from single-element and dual-element (cross-recurrence) analyses. The glmnet package (v2.0-13) in R was used to implement the model. Supplementary Table 5 lists the features that survived shrinkage during cross-validation of the model, and their associated parameter estimates.

For weighted quantile sum (WQS) regression, models followed the form:

$$g(\mu) = \beta_0 + \beta_1 \sum_j w_i q_{ij} + z'\varphi$$

Here, β_0 indicates the model intercept, *z*' and φ indicate a vector of covariates and associated regression parameters, and $\beta_1 \sum_j w_i q_{ij}$ reflects the regression parameter (β_1) and weighted quantile sum index ($\sum_j w_i q_{ij}$), where w_i indicates the estimated weight for each predictor variable and q_{ij} reflects the ranked (here, quartiles) measure per subject; the summation of these terms ($w_i q_{ij}$) provides the weighted quantile sum index. A link function, g(μ), allows the generalization of this equation to multiple distributions; for classification, as in this study, the logit link to the binomial distribution was used, i.e. $\log(\frac{\mu}{1-\mu})$, where μ is the probability of a subject diagnosed as an ASD case. All parameters, including weights, are estimated via maximal likelihood estimation, where final weights are averaged across 100 bootstrapped samples, with weights constrained to vary between 0-1, and to sum to 1.

During model training, a subset of the data are used to estimate weights for each variable, which are then used to calculate and test a WQS index in the validation dataset. The weights (training set) and parameter estimates (validation set) from these models are then applied for prediction in the naïve data in the holdout dataset. Model fit and variable weights from WQS regression are provided in Supplementary Figure 4.

Results

Population-Independent Properties of Zinc-Copper Cycles

It is possible that highly evolved zinc-copper cycles have consistent properties within a species and, therefore, would show small variances across geographic populations, similar to other physiologic set points such as normal body temperature or blood pressure. We were able to test whether copper-zinc cycles are population independent using an equivalence testing approach (*25*). This statistical method is commonly used to test for bioequivalence between the efficacy of pharmaceuticals (*25*). We found that for all three measures of zinc-copper rhythmicity, the four different populations tested were bioequivalent (Fig. S3).

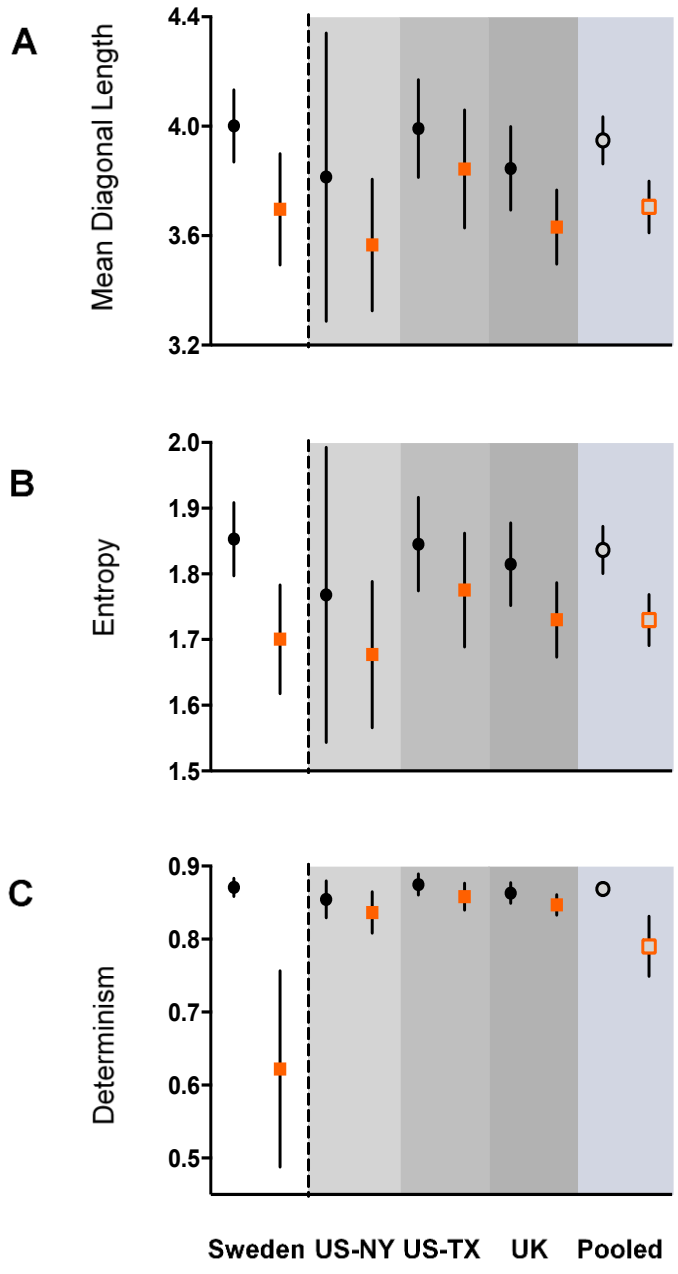
Dysregulation of Other Elemental Cycles

Earlier work has shown that lead exposure is associated with autism risk (*26*), and zinc is known to be protective against the toxic effects of lead in animal models (*27*). We observed that ASD cases exhibited zinc-lead cycles that were different to their non-ASD counterparts (Fig. S1 and Table S1). As with the zinc-copper cycles, overall cases displayed reduced determinism ($P<0.001$) and entropy ($P<0.05$), and tended to exhibit reduced MDL ($P=0.06$), but these differences were not consistent across populations, particularly for determinism.

As an exploratory analysis, we also examined cycles of individual elements (Table S2). We found that copper rhythms were elongated (higher mean diagonal length, $P=0.002$) in ASD cases and

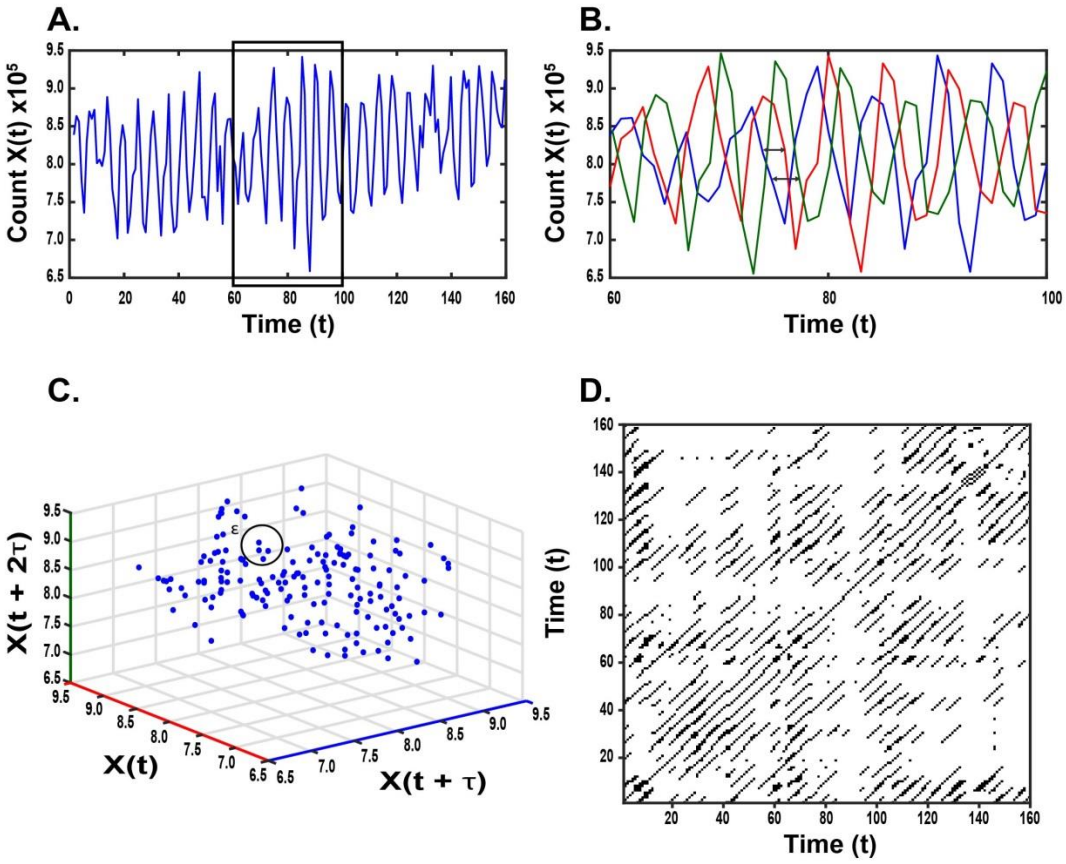
showed greater complexity (increased entropy, P=0.001) and determinism (P=0.002) in ASD cases compared to controls. Copper rhythms in ASD cases also tended to have a smaller time gap between cycles, as indicated by a reduction in recurrence time type 2 (P=0.004). A reversal in these trends was evident for lithium, where cases showed rhythms that were shorter (P=0.04), less complex (P=0.049) and unstable (P=0.022) for lithium and had reduced determinism lead (P=0.022).

Fig. S1. Disruption of Zinc-Lead Cycles in ASD.



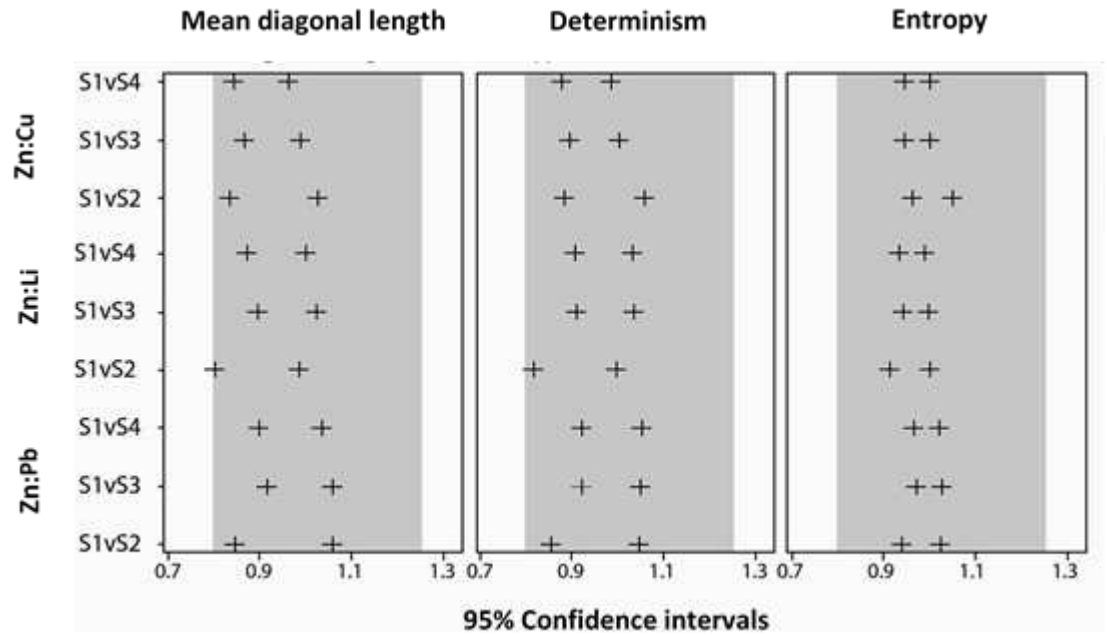
Statistically non-significant reductions in mean diagonal length (**A**), entropy (**B**) and determinism (**C**) are observed in autism cases (squares) compared to controls (circles) in all study populations. Data are means +/- 95% CI. Pooled estimates generated by combining data from all studies.

Fig. S2. Recurrence Quantification Analysis.



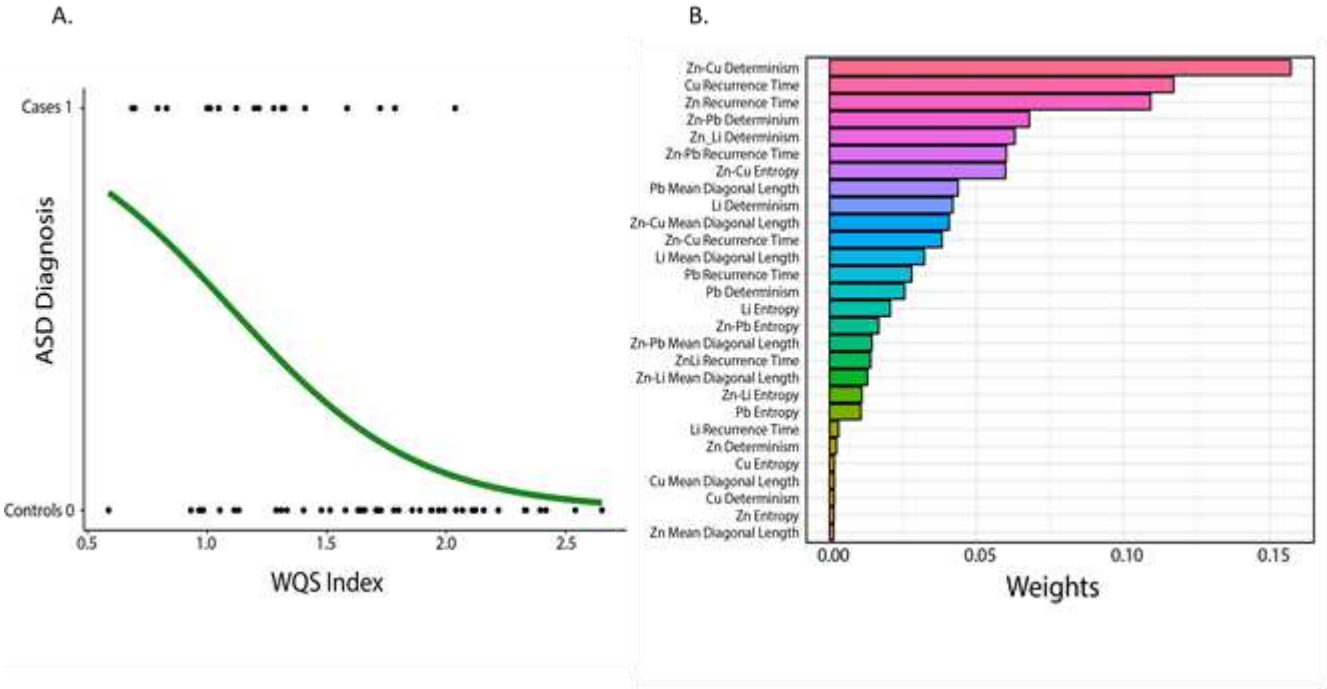
(**A**) Developmental exposure profile for Ca, expressed as counts, reflecting elemental concentrations in tooth matrices at approximately 100 sequential sampling points that reflect a time period from the second trimester to birth. (**B**) Delay embedding of the signal in (A), showing the paneled-section of the waveform. The original signal is shown in blue, with green and red lines reflecting two embeddings (time-lagged duplicates) of delay (τ) 10. (**C**) Phase-portrait of the Ca time-series in (A) plotted in three-dimensions. The coordinates of a given point are determined by the value of each delay-embedded signal at that time interval. Black circle indicates an exemplar threshold (ε) used to construct a recurrence matrix (shown in D). (**D**) Recurrence plot of the Ca time-series. Black marks in matrix indicate time-points where the system entered a given state (within-threshold on phase-portrait); e.g., all black marks vertical of day 20 indicate times when the system returned to the state it was in at day 20. Diagonal structures in this plot reflect periods of stable cyclical orbits, while white space indicates unique values, and laminar structures (vertical/horizontal lines) reflect static states. During recurrence quantification analysis (RQA), the duration and distribution of cyclical periods are quantified. Note this figure appears also in the supplementary materials to Curtin et al. (2017).

Fig. S3. Equivalence Testing of Control Group Means Across Studies.



Asymmetric family-wise 95% confidence intervals (based on Dunnett comparisons) on cross-recurrence measures for the ratio of study means (S1/S2, S1/S3 and S1/S4) of control groups, adjusted by sex. S1: Sweden, S2: Texas, S3: NY, and S4: UK. When the two family-wise confidence intervals are contained in the equivalence bounds of 0.8, 1.25 (shaded region), the four study means for the specified cross-recurrence are equivalent.

Fig. S4. Model Fit and Weight of Variables Contributing to the Weighted Quantile Sum (WQS) Regression Model.



(**A**) Model fit in the validation dataset. Performance metrics describe classification error in the validation dataset (51% of data), from which regression parameters are estimated. (**B**) Variable weights extracted from the training dataset (34% of data).

Table S1. Results of Cross-Recurrence Analyses.

	Measure	Sweden	US-NY	US-TX	UK	Pooled
Zinc:Copper	Determinism	0.001	<0.001	0.03	0.063	<0.001
	Mean Diagonal Length	<0.001	0.029	0.005	0.023	<0.001
	Entropy	<0.001	0.003	0.003	0.037	<0.001
	Recurrence Time	1	0.098	0.63	1	0.072
Zinc:Lead	Determinism	<0.001	1	1	1	<0.001
	Mean Diagonal Length	0.25	1	1	.43	0.061
	Entropy	0.067	1	1	.46	0.048
	Recurrence Time	0.93	0.96	1	.54	0.061
Zinc:Lithium	Determinism	1	1	1	1	0.73
	Mean Diagonal Length	0.69	1	1	1	0.81
	Entropy	1	1	1	1	0.65
	Recurrence Time	1	0.82	0.70	1	0.18

Values in the table reflect P values from linear mixed models regressing the effect of ASD-diagnosis (Pooled column; FDR-adjusted main effect of ASD) and study-specific posthoc tests (Bonferroni-adjusted for multiple comparisons) comparing measures between ASD cases and controls within an ASD*Study interaction.

Table S2. Results of Single Recurrence Analyses.

	Measure	Sweden	US-NY	US-TX	UK	Pooled
Copper	Determinism	0.99	0.007	0.46	1	0.002
	Mean Diagonal Length	0.4	0.037	0.63	0.27	0.002
	Entropy	0.47	0.019	0.46	0.16	0.001
	Recurrence Time	0.009	0.058	0.73	1	0.004
Lithium	Determinism	0.038	0.44	1	1	0.022
	Mean Diagonal Length	0.059	1	0.34	1	0.04
	Entropy	0.066	1	0.67	1	0.049
	Recurrence Time	1	1	1	1	0.57
Lead	Determinism	1	1	0.94	0.012	0.022
	Mean Diagonal Length	0.59	1	1	0.24	0.18
	Entropy	0.64	1	1	0.15	0.13
	Recurrence Time	0.18	1	1	1	0.57
Zinc	Determinism	1	0.80	1	1	0.44
	Mean Diagonal Length	1	0.65	0.78	1	0.21
	Entropy	1	0.70	1	1	0.39
	Recurrence Time	0.45	0.34	1	1	0.13

Values in the table reflect P values from linear mixed models regressing the main effect of ASD-diagnosis (Pooled column; FDR-adjusted main effect of ASD) and study-specific posthoc tests (Bonferroni-adjusted for multiple comparisons) comparing measures between ASD cases and controls within an ASD*Study interaction.

Table S3. Laser Ablation Analysis of Teeth.

NWR-193 Laser Conditions		**Agilent 8800 ICP-MS Conditions**	
Wavelength (nm)	193	RF power (W)	1350
Helium carrier flow (L min^{-1})	0.8	Argon carrier flow (L min^{-1})	0.6
Fluence (J cm^{-1})	5.0	Plasma gas flow (L min^{-1})	15
Repetition rate (Hz)	10	Sample Depth (mm)	4.0
Spot size (μm)	35	Scan mode	Peak hopping
Scan speed (μm s^{-1})	35	Integration time (ms)	50 – 55

Table S4. Main Effects and Interactions Across Elemental Pathways.

Elemental Pathway	RQA Feature	Effect	F Value	FDR adjusted *P*
Zn_Cu	Determinism	ASD	47.99	**7.74E-07**
		ASD*Study	2.56	0.072
Zn_Cu	Entropy	ASD	57.3	**1.18E-07**
		ASD*Study	1.98	0.140
Zn_Cu	Mean_DL	ASD	52.25	**2.63E-07**
		ASD*Study	3.83	**0.014**
Zn_Cu	RT2	ASD	4.64	0.072
		ASD*Study	1.77	0.189
Cu	Determinism	ASD	15.51	**0.002**
		ASD*Study	4.25	**0.008**
Cu	Entropy	ASD	18.2	**0.001**
		ASD*Study	5.32	**0.002**
Cu	Mean_DL	ASD	15.6	**0.002**
		ASD*Study	6.51	**0.001**
Cu	RT2	ASD	12.46	**0.004**
		ASD*Study	3.85	**0.014**
Zn_Pb	Determinism	ASD	30.21	**1.93E-05**
		ASD*Study	12.39	**8.64E-07**
Zn_Pb	Entropy	ASD	5.84	**0.048**
		ASD*Study	0.93	0.552
Zn_Pb	Mean_DL	ASD	5.03	0.061
		ASD*Study	1.4	0.295
Zn_Pb	RT2	ASD	5.05	0.061
		ASD*Study	21.68	**2.10E-09**
Pb	Determinism	ASD	7.8	**0.023**
		ASD*Study	2.42	0.076
Pb	Entropy	ASD	3.18	0.131
		ASD*Study	2.65	0.061
Pb	Mean_DL	ASD	2.46	0.180
		ASD*Study	2.99	**0.044**
Pb	RT2	ASD	0.45	0.567
		ASD*Study	0.87	0.567
Zn_Li	Determinism	ASD	0.15	0.725
		ASD*Study	2.86	**0.048**
Zn_Li	Entropy	ASD	0.26	0.649
		ASD*Study	1.43	0.289
Zn_Li	Mean_DL	ASD	0.06	0.811
		ASD*Study	2.47	0.072
Zn_Li	RT2	ASD	2.5	0.179
		ASD*Study	1.3	0.338
Li	Determinism	ASD	7.91	**0.022**
		ASD*Study	2.26	0.096
Li	Entropy	ASD	5.65	**0.050**
		ASD*Study	2.18	0.106
Li	Mean_DL	ASD	6.43	**0.040**
		ASD*Study	1.47	0.278
Li	RT2	ASD	0.41	0.567
		ASD*Study	0.56	0.772
Zn	Determinism	ASD	0.81	0.436
		ASD*Study	1.68	0.204
Zn	Entropy	ASD	0.98	0.392
		ASD*Study	4.9	**0.003**
Zn	Mean_DL	ASD	2.06	0.213
		ASD*Study	5.95	**0.001**
Zn	RT2	ASD	3.14	0.131
		ASD*Study	10.5	**5.20E-06**

For each elemental pathway or cross-recurrence (column 1), the effects of ASD diagnosis or an ASD*Study interaction (rows in effect column) were tested on 4 RQA features. Test statistics and FDR-adjusted *P* values are given for each effect. FDR adjustment reflects the 56 tests done across all pathways and effects listed.

Table S5. Features Preserved in the Penalized Logistic Regression Classifier.

Elemental pathway	RQA Feature	Beta
Cu	Determinism	2.0604
Cu	Entropy	4.0472
Cu	Mean Diagonal Length	.
Cu	RT2	-0.062
Li	Determinism	.
Li	Entropy	.
Li	Mean Diagonal Length	-0.653
Li	RT2	.
Pb	Determinism	-5.623
Pb	Entropy	.
Pb	Mean Diagonal Length	.
Pb	RT2	.
Zn	Determinism	14.538
Zn	Entropy	.
Zn	Mean Diagonal Length	0.8691
Zn	RT2	-0.023
Zn_Cu	Determinism	-13.89
Zn_Cu	Entropy	-1.295
Zn_Cu	Mean Diagonal Length	-3.159
Zn_Cu	RT2	-0.298
Zn_Li	Determinism	-0.746
Zn_Li	Entropy	-0.658
Zn_Li	Mean Diagonal Length	.
Zn_Li	RT2	-0.169
Zn_Pb	Determinism	-3.683
Zn_Pb	Entropy	.
Zn_Pb	Mean Diagonal Length	.
Zn_Pb	RT2	.

All features included during cross-validation and prediction are listed by elemental pathway (column 1) and dynamical feature extracted via recurrence quantification analysis (RQA) or cross-recurrence quantification analysis (CRQA) (column 2). Associated beta estimates are provided in column 3, with blank values (.) indicating features zeroed during LASSO shrinkage.

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